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The effects of age and gender on parathyroid hormone dynamics

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Summary

BACKGROUND AND AIMS Hyperparathyroidism is a risk factor for bone loss. An age-related increase in parathyroid hormone (PTH) level has been demonstrated in several studies. It has been suggested that the type II osteoporotic syndrome, a condition of increased prevalence among elderly women, may be at least partially caused by elevations in intact parathyroid hormone (iPTH) levels. To date, however, the effects of age and gender *per se* on PTH dynamics in healthy subjects independent of other risk factors such as vitamin D deficiency and/or impaired renal function that can impact on parathyroid function, remain unknown. In this study, we used citrate and calcium (Ca) infusions to characterize the impact of age and gender on PTH dynamics in normal subjects.

SUBJECTS AND METHODS Twelve young women with mean age ± SD of 26·4 ± 1·6 years, 12 young men with mean age of 26·6 ± 1·3 years, 12 older women with mean age of 68·6 ± 1·3 years and 12 older men with mean age of 67·2 ± 1·6 years were studied. The sigmoidal curves relating serum iPTH to serum levels of ionized Ca (Ca_i) were characterized by maximal and minimal iPTH levels, the set-points (levels of Ca_i causing half-maximal suppression of iPTH), and the slopes of the curves at the set-points.

RESULTS Baseline serum Ca, Ca_i, 25 hydroxyvitamin D [25(OH)D] and 1,25 dihydroxyvitamin D [$1,25(OH)_2D_3$] levels, as well as the set-points, slopes and minimal values of the sigmoidal curves relating Ca_i to iPTH, did not differ among the four groups. iPTH levels at baseline were slightly but not significantly higher in the older age groups ($P = 0{\cdot}18$). The maximal iPTH level was 25% higher in the older women than in the younger women, although this difference was not significant ($P = 0{\cdot}29$). However, the integrated iPTH responses calculated from the areas under the curves (AUC) of iPTH levels *vs.* time during the calcium and citrate infusions were significantly higher in postmenopausal women than in young women during both infusions and in older men than in young men during the calcium infusion. There was no effect of gender on serum iPTH levels.

CONCLUSIONS In both women and men, ageing *per se*, independent of changes in vitamin D economy or renal function, is associated with an increase in integrated PTH secretory response to changes in serum calcium. No alterations in the Ca_i/iPTH set-point were present. The biological relevance of these modest increments in integrated iPTH levels during dynamic testing in older healthy men and women remain uncertain.

Maximal bone mass is achieved between 20 and 40 years of age, after which bone loss occurs in both genders. Over a lifetime, women lose 50% of their trabecular bone mass and 30% of their cortical bone mass, whereas men lose two-thirds of these amounts (Riggs *et al*., 1981; Mazess, 1990; Riggs & Melton, 1992). Bone loss with ageing is the result of complex interactions of multiple factors, including genetic traits, environmental variables, diet, physical activity, gonadal status and calciotropic hormones (Sherman *et al*., 1990; Silverberg *et al*., 1989; Hernandez-Avila *et al*., 1991; Cauley *et al*., 1995; Cummings *et al*., 1995; Silverberg *et al*., 1995). Because of the significant impact of parathyroid hormone (iPTH) on calcium (Ca) and skeletal homeostasis, investigators have evaluated serum iPTH levels in elderly subjects and have demonstrated significant increments with age (Kotowicz *et al*., 1990; Orwoll & Meier, 1986; Sherman *et al*., 1992; Prince *et al*., 1995; Khosla *et al*., 1997). These increments in baseline serum iPTH levels may play an important role in age-related bone loss (Orwoll & Meier, 1986; Sherman *et al*., 1992). Indeed, several studies have shown an inverse relationship between iPTH levels and bone mass at several skeletal sites (Orwoll & Meier, 1986; Benhamou *et al*., 1991; Villareal *et al*., 1991; Sherman *et al*., 1992). Likewise, even mild primary hyperparathyroidism is usually accompanied by some bone loss, particularly cortical bone (Silverberg *et al*., 1989).

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The mechanisms thought to underlie the age-related increase in serum iPTH levels include decreased renal clearance (Fajtova *et al*., 1995), secondary hyperparathyroidism due to hypovitaminosis D, low dietary calcium intake, and/or gut resistance to vitamin D action (Riggs & Melton, 1986; Chapuy *et al*., 1992; Dawson-Hughes *et al*., 1995; Gloth *et al*., 1995; Ledger *et al*., 1995). Abnormalities in vitamin D levels and/or action are the best recognized cause of age-related increments in iPTH levels. Elevations in iPTH levels can be reversed with vitamin D supplementation (Chapuy *et al*., 1992; Dawson-Hughes *et al*., 1995). iPTH levels are mildly elevated in patients with hip fracture (Benhamou *et al*., 1991) and in one study were decreased by 44% upon supplementation with vitamin D in conjunction with a reduction in fracture incidence (Chapuy *et al*., 1992). However, these elevations in iPTH levels may not reflect the normal ageing process in a healthy population (Sherman *et al*., 1992; Harris *et al*., 1993), and whether ageing *per se* contributes to the age-related increase in iPTH, independent of these other comorbid factors, remains unclear.

Investigations evaluating the impact of ageing on serum iPTH levels that have used measurements of random baseline levels (Kotowicz *et al*., 1990; Orwoll & Meier, 1986; Sherman *et al*., 1992; Khosla *et al*., 1997) do not adequately reflect iPTH dynamics, since baseline iPTH levels can be influenced by diet, exercise and diurnal variations (El-Hajj Fuleihan *et al*., 1997). Therefore, a more complete assessment of parathyroid gland function using dynamic studies that assess the complete Ca_i/iPTH relationship is needed. Indeed, systematic evaluation of this axis has revealed subtle abnormalities in serum iPTH secretion in African-American subjects (El-Hajj Fuleihan *et al*., 1994) that may not be detected by measurement of baseline values (Meier *et al*., 1991; Dawson-Hughes *et al*., 1993).

Ionized calcium (Ca_i) tightly regulates iPTH release. The Ca_i/iPTH relationship is characterized by a sigmoidal curve defined by four parameters: maximal iPTH response to hypocalcemia, maximal iPTH suppression in response to hypercalcemia, the set-point (the Ca_i concentration at which iPTH suppression is half-maximal), and the slope at the set-point. An increase in any one of these parameters can produce hypersecretion of iPTH at a given level of Ca_i (Brown, 1983).

Previous work has shown that factors associated with ageing can impact on iPTH dynamics as well as on baseline iPTH levels. In a study evaluating iPTH dynamics in elderly subjects, Ledger *et al*. (1994) demonstrated an increase in serum iPTH levels that was reversed by 1,25 dihydroxyvitamin D [$1,25(OH)_2D_3$] administration. However, these subjects had 25 hydroxyvitamin D [25(OH)D] levels at the lower end of the normal range, and it is unclear whether this observation can be generalized to healthy elderly people who are not vitamin D insufficient. Portale *et al*. (1997) reported a shift in the set-point to the right in older healthy men compared to younger men, with a 20% increase in maximal iPTH levels in the older age group.

Osteoporosis is more prevalent among women than among men. Abnormalities in iPTH secretion play an important role in bone loss. Thus, hypersecretion of iPTH might be expected to be more pronounced in women than in men. Alterations in PTH dynamics between genders and osteoporotic and normal subjects have been noted previously. Indeed, two studies have demonstrated a greater amplitude for PTH diurnal rhythm in men than in women and in normal than in osteoporotic subjects (Calvo *et al*., 1991; Eastell *et al*., 1992). Data in a small number of male subjects suffering from osteoporosis suggests a decrease in PTH pulse amplitude and frequency when compared to young-matched subjects (Harms *et al*., 1989). However, to our knowledge, no studies have evaluated the impact of age and gender on PTH dynamics. Therefore, in this study, we systematically evaluated the impact of both age and gender on iPTH dynamics in normal healthy individuals independent of age-related changes in vitamin D economy and renal function.

Materials and methods

Subjects

Twelve subjects in each of four categories: premenopausal women, postmenopausal women, young men (age range between 20 and 40 years), and older men (age range between 60 and 80 years) were each studied over 2 days. Our subject number of 12 per group was based on an initial pilot study and the data from Ledger *et al*. (1994) as estimated to detect a 20 ng/l difference in maximal iPTH release between young and old subjects within each gender (power 80%, $P = 0{\cdot}05$). All subjects were Caucasian to eliminate any ethnic differences in iPTH levels (El-Hajj Fuleihan *et al*., 1994). The subjects were recruited through IRB-approved advertisements placed in local newspapers and on campus noticeboards. Twenty subjects received a Ca infusion on the first day followed by a citrate infusion on the second day, whereas 28 subjects received the citrate infusion first and the Ca infusion second. Because of the effect of the menstrual cycle on serum iPTH levels (El-Hajj Fuleihan *et al*., 1993), young women had to have spontaneous, regular cycles of 28–35 days as documented by a 3 month diary to be eligible for participation and were studied during the follicular phase (first 7 days). The postmenopausal women were not taking hormone replacement therapy or any other medication known to affect bone metabolism. Subjects had to have bone mineral densities (BMD) of the lumbar spine and femoral neck that were within 2 SD of those for age-and gender-matched controls. Before enrolment each subject underwent a physical examination and a laboratory evaluation that included a multichannel serum chemistry analysis and a complete blood

count with differential, a serum iPTH level, a serum thyroid-stimulating hormone (TSH) level, and a serum 25(OH)D level. Subjects with vitamin D insufficiency [25(OH)D levels below 37·4 nmol/l] were excluded (Gloth *et al*., 1995) and all subjects had serum levels of urea and creatinine that were within the normal range. To avoid any confounding effect of obesity on serum iPTH levels (Bell *et al*., 1985), all subjects had a body mass index (BMI, weight in kg divided by height in metres squared) of less than 30. The subject's mean dietary Ca intake was evaluated with a Ca questionnaire generated by the General Clinical Research Center dietitian. The study was reviewed and approved by the Committee for the Protection of Human Subjects of the Brigham and Women's Hospital. Informed written consent was obtained from each subject before their participation in the study.

Study design

The design of the protocol has been described in detail in previous publications examining PTH dynamics in healthy volunteers (Grant *et al*., 1990; El-Hajj Fuleihan *et al*., 1994). The protocol required two visits to the Ambulatory Clinical Center, one for a citrate infusion and the other for a Ca infusion. For each visit, the subject arrived at 0800 h in a fasting state. An intravenous catheter was placed in a vein of each antecubital fossa and kept open with 5% dextrose in water (D5W). One intravenous line was used for blood sampling and the other for infusion of Ca or citrate. Samples for the determination of serum $1,25(OH)_2D_3$ levels and for chemistry analyses, including measurements of blood urea and creatinine (Cr), were obtained before the initiation and upon completion of each infusion. Serum 25(OH)D levels were measured at the beginning of the infusion on the first day only.

Citrate infusion

Citrate (anticoagulant-citrate-dextrose USP Formula A [ACD-A] containing per 100 ml: 2·45 g dextrose, 2·2 g sodium citrate, and 0·7 g citric acid; Fenwal Laboratories, Deerfield, IL, USA) mixed in 5% dextrose water was administered via an IV infusion pump (Travenol, Deerfield, IL, USA). Throughout the course of the infusion, blood pressure was monitored by an automated blood pressure recorder (adult/paediatric vital sign monitor, Critikon, Inc., Tampa, FL, USA), and an EKG was obtained from a cardiac monitor (Physio Control Lifepak 7, Rowayton, CT, USA) at each step before the infusion rate was increased. The study consisted of four 30 min pulse-step intervals of citrate infusion. In brief, a rapid 5 min infusion of citrate was followed by a slower infusion for 25 min. Progressively increasing rates of both the fast and slow infusions were used for three additional 30 min periods. The citrate dose was 42 mg/kg/h, followed by 20 mg/kg/h, for the first 30 min interval; dosages for subsequent intervals were 70/33, 96/44, and 130/60 mg/kg/h, respectively. iPTH levels measured at 5 min after the beginning of each step reflect the effect of the initial, rapid change in Ca_i on iPTH release, whereas the 30 min points, when Ca_i is more stable, more closely reflect the effect of Ca_i concentration *per se* on iPTH dynamics (Grant *et al*., 1990). Samples for measurement of serum Ca_i and iPTH levels were collected at 0, 5, 10, 20 and 30 min during each step of the infusion.

Calcium infusion

Calcium gluconate (Astra, Westboro, MA, USA) was infused over three 30 min pulse-step intervals via an IV infusion pump by the same procedure as that used for the citrate. The doses for Ca for the fast/slow infusions were 2·4 mg/kg/h, followed by 0·75 mg/kg/h, in step 1; 3·4 mg/kg/h, followed by 1·25 mg/kg/h, in step 2; and 4·4 mg/kg/hr, followed by 1·75 mg/kg/h, in step 3. Samples for serum Ca_i and iPTH levels were collected at 0, 5, 10, 20 and 30 min for each step of the infusion.

Characterization of the Ca_i-iPTH relationship

The following indices of parathyroid function were used to evaluate the relationship of iPTH to Ca_i: the maximal iPTH response to hypocalcemia (max. PTH), the minimal iPTH level achieved during induced hypercalcemia (min. PTH), the set-point for iPTH-defined as the serum Ca_i concentration at which the iPTH level is half maximally suppressed-and the slope of the curve at the set-point (Brown, 1983). Samples obtained at the 30 min time points of the Ca and citrate infusions, when Ca_i levels were stable, were used to characterize the Ca_i-iPTH axis. Sigmoidal curves were fit with GraphPad Prism software 1·0 (GraphPad Software, San Diego, CA, USA).

Laboratory tests

Serum levels of Ca, phosphate (PO_4), magnesium (Mg), and Cr were determined by the clinical chemistry laboratory by a colourimetric method with an Olympus AU-5061 analyser (Olympus Corporation, Lake Success, NY, USA) at the main laboratory of the Brigham and Women's Hospital. Blood for serum Ca_i was collected anaerobically and measured with an AVL 987-S electrolyte analyser (AVL Scientific Corporation, Roswell, GA, USA), which has an intra-assay precision of 0·39% and an interassay precision of 1·7–2·5% for Ca_i levels between 1·12 and 1·48 mmol/l. In our laboratory, the normal range (derived from 57 normal healthy subjects) is 1·15–1·33 mmol/l. Fasting urinary Ca and Cr levels were measured on both days before administration of the infusion.

Table 1 Baseline characteristics of study subjects

	Young women ($n = 12$)	Older women ($n = 12$)	Young men ($n = 12$)	Older men ($n = 12$)
Age (years ± SD)	26·4 ± 1·6	68·6 ± 1·3	26·6 ± 1·3	67·2 ± 1·6
Body mass index (kg/m^2)	23·2 ± 0·5	24·3 ± 0·9	25·0 ± 0·8	26·6 ± 0·8
Dietary calcium (mg/day)	1050 ± 157	1283 ± 115	1235 ± 221	896 ± 111
Serum calcium (mmol/l)	2·45 ± 0·03	2·44 ± 0·03	2·47 ± 0·03	2·49 ± 0·03
Serum creatinine (μmol/l)	85·7 ± 2·7	91·9 ± 2·7	99·9 ± 4·4	86·6 ± 5·3
Intact parathyroid hormone (ng/l)	27·0 ± 3·7	34·9 ± 3·3	30·4 ± 3·7	31·7 ± 2·8
Ionized calcium (mmol/l)*	1·24 ± 0·01	1·24 ± 0·01	1·25 ± 0·01	1·24 ± 0·01
25 (OH)D (nmol/l)*	63·4 ± 4·2	61·7 ± 5·0	64·4 ± 6·2	62·9 ± 3·0
1,25 $(OH)_2D_3$ (pmol/l)*	75·4 ± 8·6	93·0 ± 7·0	84·0 ± 6·5	81·8 ± 6·7
Urinary Ca/Cr*	0·09 ± 0·01	0·09 ± 0·01	0·08 ± 0·02	0·08 ± 0·02

* Obtained from day 1 infusion. Measurements expressed as mean ± SEM.

Serum iPTH was measured by the Allegro immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA, USA) (Nussbaum *et al*., 1987). The detection limit of the assay is 1·0 ng/l (normal range: 10–65 ng/l); and the intra-and inter-assay CV%s are 1·7% and 6·5% at iPTH concentrations of 37·7 and 44·1 ng/l, respectively. Serum 25(OH)D was measured by a competitive protein-binding assay (Incstar, Stillwater, MN, USA) with the normal range of 25–137 mmol/l. A radioreceptor assay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA), which utilizes the 1,25$(OH)_2D_3$ receptor extracted from calf thymus, was used for the 1,25$(OH)_2D_3$ assay (normal range 36–156 pmol/l). For 25(OH)D, the intra-assay CV%s are 8·7% and 8·9% at serum concentrations of 30 and 132 nmol/l, respectively, and the interassay CV% is 12% at a serum concentration of 122 nmol/l. For 1,25$(OH)_2D_3$, the intra-assay CV% is 8·7% at a concentration of 82 pmol/l, and the interassay CV% is 13·2% at a concentration of 103 pmol/l. Except for serum Ca_i samples, which were measured on the same day, all other serum samples were stored at −70°C. All samples from each patient (except for Ca_i) were run in duplicate and in the same assay.

BMDs of the spine (L1-L4), hip (femoral neck, trochanter) and forearm (distal one-third radius) were measured in all subjects with a dual-energy X-ray absorptiometer (DEXA), QDR 2000 (quantitative digital radiography, Hologic, Inc., Waltham, MA, USA), to ensure that all subjects were within 2 SD of age-and gender-matched controls. BMD was expressed in both groups as *Z*-scores, the number of standard deviations compared with an age-matched normative database supplied by the software for the bone densitometer.

Statistical analysis

Baseline laboratory studies were analysed by analysis of variance (ANOVA) with factors of age group, gender, and the interaction between age group and gender. In addition, four indices of parathyroid function were calculated: Maximal responsiveness (PTH max), maximal suppressibility (PTH min), the set-point, and the slope of the curve at the set-point. Set-points were calculated by two methods. In Method I, the levels of iPTH at the 30 min time points for the citrate and Ca infusions were averaged for each study group, and a sigmoidal curve was fitted to the average data points for each group. In Method II, a sigmoidal curve was fitted to each individual's data and the four indices were derived from the individual curves. The individual subjects' set-points were then averaged to calculate a mean set-point for each study group. The indices of parathyroid function were also analysed by ANOVA to test for the effects of the following variables: age groups, gender and the interaction of these two variables. The data from both infusions (i.e. Ca_i and iPTH) also included multiple measures sampled repeatedly over time. The integrated iPTH response was estimated by the sum of the discrete areas of the trapezoids formed by the iPTH levels at consecutive time points. The focus of this analysis of the integrated iPTH responses was on the effect of age within each gender. Therefore, in addition to the main effect from the ANOVA, the corresponding simple effects are reported (Kirk, 1982). The results are expressed as mean ± SEM, unless otherwise indicated. Significance was indicated for $P < 0{\cdot}05$. Reported *P*-values are not adjusted for multiple tests.

Results

Characteristics prior to infusion at screening and at time zero of each infusion

Table 1 shows baseline characteristics for the four groups.

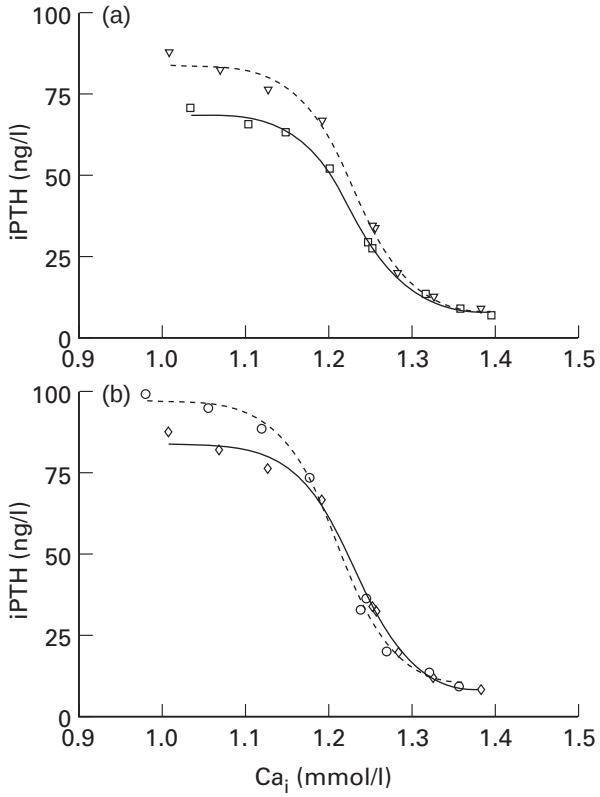

Fig. 1 The age effect on parathyroid hormone (PTH) dynamics in (a) Young and older men □ Young men (set-point = 1·23 mmol/l); ▽ Older men (set-point = 1·23 mmol/l) and (b) Young and older women ◇ Young women (set-point = 1·22 mmol/l); ○ Older women (set-point = 1·20 mmol/l). Sigmoidal curves were generated from the 30 min data points when serum Ca_i levels were stable during the citrate and Ca infusions.

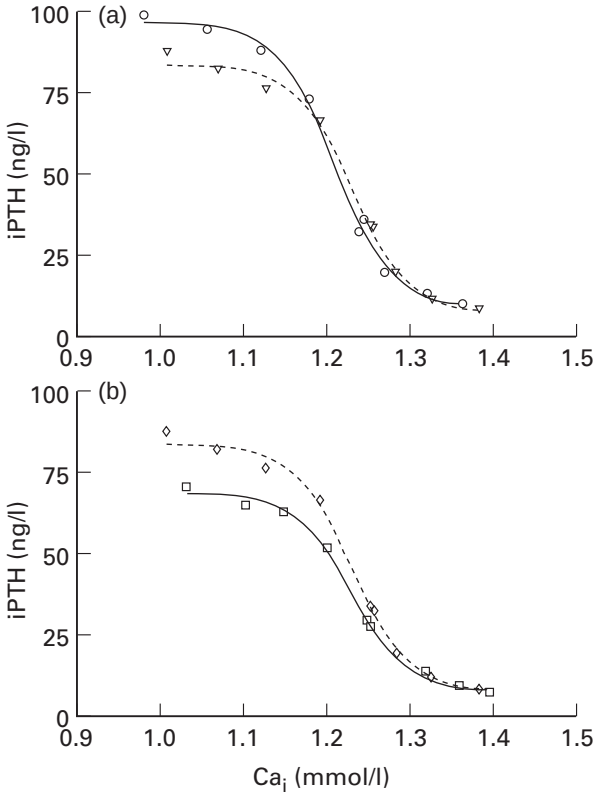

Fig. 2 The gender effect on parathyroid hormone (PTH) dynamics in (a) Older women and men ○ Older women (set-point = 1·20 mmol/l); ▽ Older men (set-point = 1·23 mmol/l) and (b) Young women and men ◇ Young women (set-point = 1·22 mmol/l); □ Young men (set-point = 1·23 mmol/l). Sigmoidal curves were generated from the 30 min data points when serum Ca_i levels were stable during the citrate and Ca infusions.

The four study groups did not differ significantly in terms of dietary Ca intake. Mean baseline serum levels of Ca, Ca_i, 25(OH)D, and $1,25(OH)_2D_3$ and mean urinary levels of Ca and urine Ca/Cr at the screening visit and at time zero of the infusions were not significantly different among the four groups. The screening mean iPTH levels were slightly higher in the older groups, ($P = 0{\cdot}18$). Two premenopausal women, despite having serum 25(OH)D levels above 50 nmol/l, had serum iPTH levels of 50 and 48 ng/l, respectively. In our laboratory, the mean serum iPTH level at baseline in young women is 32 ± 4 ng/l (Brent *et al*., 1988). In addition, the older age groups had higher iPTH levels compared to the younger subjects on both the citrate ($32{\cdot}5 \pm 8{\cdot}0$ ng/l *vs.* $27{\cdot}3 \pm 11{\cdot}5$ ng/l) ($P = 0{\cdot}01$) and calcium days ($36{\cdot}2 \pm 11{\cdot}5$ ng/l *vs.* $29{\cdot}2 \pm 11{\cdot}0$ ng/l) at time zero ($P = 0{\cdot}004$), respectively, but there was no significant effect of gender on serum iPTH levels. The premenopausal women had menstrual cycles lasting $29 \pm 0{\cdot}6$ days (mean ± SD). The postmenopausal women were $20{\cdot}3 \pm 1{\cdot}3$ years (mean ± SD) after the menopause.

Sigmoidal curve characteristics

To evaluate the effect of changing Ca_i concentration *per se* rather than the effect of the rate of change in Ca_i on iPTH levels, the 30 min time points from both the citrate and the Ca infusions were used to plot sigmoidal curves (Grant *et al*., 1990). The 30 min time points reflect stable serum Ca_i levels following the increases in infusion rates. As shown in Figs. 1 and 2, there were no differences in the set-point between age and gender groups. The set-points derived by Method I were as follows: premenopausal women, 1·22 mmol/l; postmenopausal women, 1·20 mmol/l; young men, 1·23 mmol/l; and older men, 1·23 mmol/l. Table 2

Table 2 Characteristics of the sigmoidal curves

	Young women ($n=12$)	Older women ($n=12$)	Young men ($n=12$)	Older men ($n=12$)
Set-point (mmol/l)	1·22 ± 0·01	1·20 ± 0·01	1·21 ± 0·03	1·22 ± 0·00
Slope of set-point	−10·6 ± 1·8	−20·5 ± 8·6	−12·6 ± 2·5	−10·5 ± 3·2
PTH max. (ng/l)	79·5 ± 7·5	99·5 ± 10·4	85·4 ± 19·5	90·5 ± 3·2
PTH min (ng/l)	4·4 ± 2·2	8·0 ± 2·7	4·6 ± 2·0	2·3 ± 4·3

* Set-point determined by Method II (see Methods section). Measurements expressed as mean ± SEM.

presents the corresponding set-points using Method II: premenopausal women, 1·22 ± 0·01 mmol/l; postmenopausal women, 1·20 ± 0·01 mmol/l; young men, 1·21 ± 0·03 mmol/l; and older men, 1·22 ± 0·00 mmol/l. The maximal iPTH responses were 79·5 ± 7·5 ng/l in the younger women, 99·5 ± 10·4 ng/l in the older women ($P=0·29$). The maximal iPTH responses were 85·4 ± 19·5 ng/l in the young men and 90·5 ± 3·2 ng/l in the older men. None of these values differed significantly from one another. The minimal iPTH levels across the groups were similar ($P=0·81$).

iPTH responsiveness to induced hypocalcemia and hypercalcemia

Analysis of the integrated iPTH measurements obtained from the iPTH *vs.* time curves during the citrate infusion showed that serum iPTH levels in the combined groups of older subjects were significantly higher than those in the young subjects ($P=0·01$) (Figs. 3 and 4). Table 3 shows that this difference in iPTH levels with age during the citrate infusion was significant in women when analysed separately ($P=0·04$), but not in men ($P=0·11$). The integrated iPTH measurements as a function of time during the calcium infusion were also significantly higher in the combined older subjects ($P<0·004$), and this effect of age was present in both women and men when analysed separately ($P=0·04$ and $P=0·03$, respectively). There were no differences between the iPTH levels in young men and women or between those in older men and postmenopausal women during either the calcium or citrate infusions ($P=0·22$ and $P=0·74$, respectively).

Discussion

This study compared the Ca_i/iPTH axis in young and older men and women by dynamic testing and has found no effect of age and gender on the set-point, slope at the set-point, maximal or minimal iPTH levels during induced hypo- and/or hypercalcemia in healthy subjects. A statistically significant, albeit modest change, however, was apparent when using an integrated measure of changes in iPTH as a function of time for the older subjects. While the older women had maximal iPTH values for the four parameter sigmoidal curves that were approximately 25% higher than in young women, this difference was not statistically significant because of the smaller number of measurements (only the 30 min time-points were used) and the substantial interindividual variability in iPTH responses). The same factors likely precluded demonstrating a significant increase in the minimal iPTH level of these curves for older *vs.* younger women despite the nearly twofold difference in this parameter (8·0 *vs.* 4·4 ng/l).

Similar observations have been made by Ledger *et al*. (1994) using a somewhat different protocol to induce hypocalcemia, although the higher levels of both maximal and minimal

Table 3 Comparison of intact parathyroid hormone (iPTH) values in the four groups during the infusions

	Integrated iPTH measurements		
	Mean	SD	*P* value
Citrate day			
Young women ($n=7$)	9112	2861	0·04
Old women ($n=11$)	12091	3770	
Young men ($n=12$)	8473	2999	0·11
Old men ($n=11$)	10438	1356	
Calcium day			
Young women ($n=7$)	1070	467	0·04
Old women ($n=11$)	1416	298	
Young men ($n=12$)	1026	288	0·03
Old men ($n=12$)	1386	471	

No adjustments were made for baseline group differences. Integrated iPTH measurements were obtained only from those patients with complete data sets, i.e. all 17 time-points during the citrate infusion, and 13 time-points during the calcium infusion.

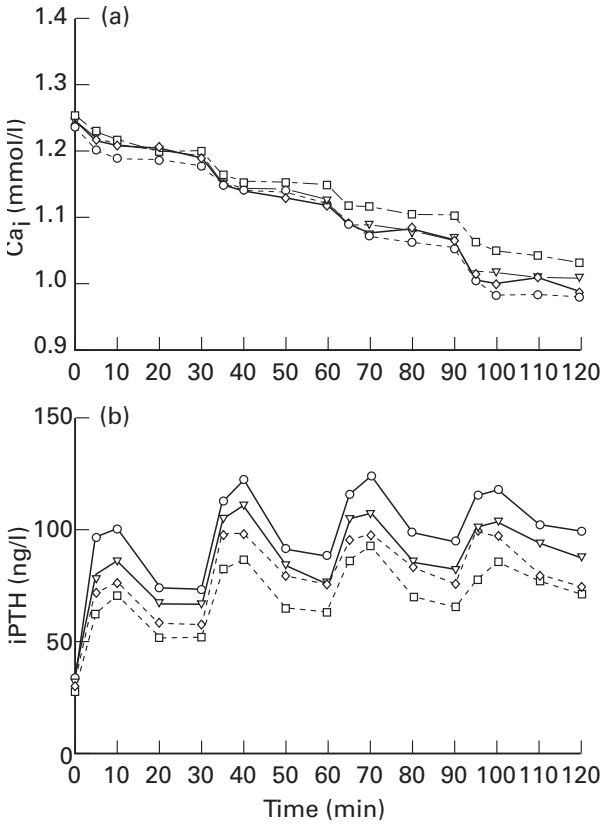

Fig. 3 Levels of (a) serum Ca_i and (b) serum intact parathyroid hormone (iPTH), obtained during the 120 min citrate infusion in the four groups. ◇ Young women; ○ Older women; □ Young men; ▽ Older men. The areas under the curves (AUC) were higher in the older men and postmenopausal women than in the younger men and premenopausal women ($P = 0{\cdot}01$).

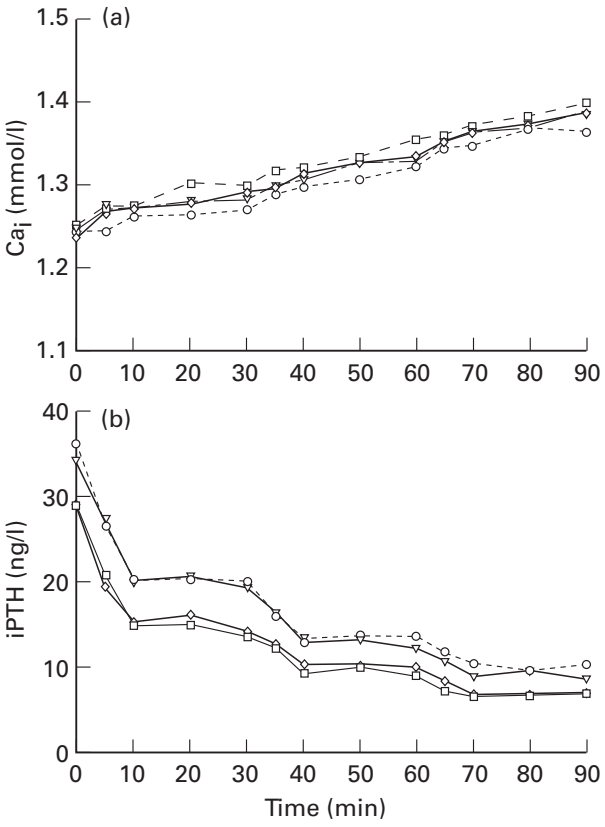

Fig. 4 Levels of (a) serum Ca_i and (b) serum intact parathyroid hormone (iPTH), obtained during the 90-minutes calcium infusion in the four groups. ◇ Young women; ○ Older women; □ Young men; ▽ Older men. The areas under the curves (AUC) in the older men and postmenopausal women were higher than in the young men and premenopausal women ($P = 0{\cdot}004$).

iPTH levels in elderly subjects in this study were caused, to a large extent, by vitamin D deficiency, as they were corrected following supplementation with $1,25(OH)_2D_3$. Our subjects all had 25(OH)D levels within the normal range, including the two young women with serum iPTH levels within the upper part of the normal range. Recent studies, however, have shown that basal iPTH levels begin to rise as vitamin D levels fall below 62 nmol/l (Chapuy *et al*., 1992; Dawson-Hughes *et al*., 1995; Haden *et al*., 1999). However, there were no differences in mean serum 25(OH)D levels between our older and younger subjects. Thus, it is unlikely that subclinical vitamin D deficiency was the cause of the higher integrated iPTH levels in our older subjects during the calcium and/or citrate infusions in our study.

A number of previous studies have demonstrated changes in basal levels of serum iPTH with increasing age (Orwoll & Meier, 1986; Kotowicz *et al*., 1990; Sherman *et al*., 1992; Prince *et al*., 1995; Khosla *et al*., 1997). This may be partially due to 'subclinical' vitamin D deficiency and the existence of other comorbid conditions that were excluded in our study. Another comorbid condition that may contribute to the elevations in basal iPTH values that have been observed in the ageing population is progressive renal deterioration, as determined by creatinine clearance, which is inversely correlated with iPTH levels (Fajtova *et al*., 1995). In addition, iPTH levels have been correlated with increasing serum creatinine levels (Yendt *et al*., 1991; Yendt *et al*., 1993). We excluded any patient with deteriorating renal function from our study, thus eliminating any possible effect of this variable on serum iPTH levels either at baseline or during induced hypo- and hyper-calcemia. Nevertheless, despite the lack of differences in the screening of basal serum iPTH levels in older *vs.* young men and women, we detected significantly higher levels of serum iPTH in older *vs.* younger subjects for the zero time

values of both the citrate and calcium infusions. Therefore, probably because of the small numbers of subjects in each of our groups (12 individuals), we were not always able to document a relatively small, but likely to be significant increase in intact serum iPTH levels (25%) in older persons, occurring independent of differences in vitamin D levels or renal function.

It has also been suggested that the iPTH assay used in our study detects not only the biologically active PTH (1–84) moiety (75–85%), but also a nonintact form of PTH (15–25%) (Brossard *et al*., 1996). If this is indeed correct, this factor may have contributed to our inability to detect very subtle differences in the truly biologically active PTH molecule between the various groups studied. However, despite excluding several factors contributing to age-related increases in basal iPTH levels, even healthy ageing does appear to be associated with modest (25–30%) increases in integrated iPTH values during induced hypo- and hyper-calcemia but with smaller changes in baseline serum iPTH levels (16%). The biological relevance of these elevated basal iPTH levels and the evoked iPTH responses with age in otherwise healthy individuals with normal vitamin D levels and renal function is unclear and requires further understanding.

Portale *et al*. (1997) have demonstrated a shift in the set-point to the right for PTH dynamics in older men compared to younger men, with a 20% increase in maximum PTH concentration. Several differences between this study and Portale *et al*. may however, explain the different results. The basal iPTH levels in the Portale study were 42 ± 4 ng/l in the older men *vs.* $31{\cdot}7 \pm 2{\cdot}8$ ng/l in our study (Portale *et al*., 1997). Moreover, the variability in 25(OH)D levels in the study of Portale *et al*. suggests that some of the older men may have been vitamin D insufficient, thus accounting for the serum iPTH levels in that group. Furthermore, differences in the experimental design may also explain the differences in results between our study and that of Portale *et al*. (Portale *et al*., 1997). The protocols used in the latter study (Portale *et al*., 1997) also did not employ the pulse step approach with the 30 min stable time points that we used in this study, thus making it hard to compare results directly. Finally, EDTA was used as a hypocalcemic agent, which causes more lowering of Ca_i than the citrate we used in our study. The absence of a change in set-points with ageing demonstrated in our study is, however, consistent with the findings of Ledger *et al*. (1994).

In conclusion, our dynamic studies demonstrate no effect of age and gender on the characteristics of the Ca_i/iPTH curve, although there was a trend toward higher maximal and minimal iPTH levels in the older women. However, our results document that integrated levels of iPTH during dynamic testing are modestly higher in older subjects compared to younger subjects. An effect of gender on iPTH levels, in contrast, was not apparent. Thus, in otherwise healthy elderly subjects, the biological relevance of these higher integrated levels of iPTH requires further investigation with respect to age-related bone loss.

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